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ACTION OF HYDROSTATIC PRESSURE ON SEA URCHIN CILIA[1]

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Hydrostatic pressure has been shown to affect organized cellular structures and macromolecular synthesis (Zimmerman, 1970). In several different species of protozoa (Kitching, 1957, 1970) and in marine tissue (Flugel and Schlieper, 1970), it has been reported that ciliary activity is inhibited by pressure. Pressure causes the disappearance of microtubular arrays in structures such as the mitotic apparatus (Zimmerman and Marsland, 1964; Zimmerman and Philpott, unpublished) and the axopodia of *Actinosphaerium* (Tilney, Hiramoto and Marsland, 1966). In *Tetrahymena* the proximal portions of the central ciliary fibers and longitudinal microtubules are affected by pressures of 7500–10,000 psi (Kennedy and Zimmerman, 1970). However, fine structural analysis of the cilia and sperm flagellae of sea urchin embryos indicate that these structures are resistant to pressure treatment (Tilney and Gibbins, 1968, 1969; Marsland, 1970).

In protozoa, protein synthesis is essential for complete flagellar and cilia regeneration (Rosenbaum and Child, 1967; Rosenbaum, Moulder and Ringo, 1969); however, RNA and protein synthesis are not necessary for initial cilia formation and regeneration in sea urchin embryos (Auclair and Meisner, 1965; Auclair and Siegel, 1966). The sea urchin thus provides a relatively simple system for studying processes involved in the organization and assembly of the cilium.

The experiments reported here were designed to investigate the effects of hydrostatic pressure on both the intact cilium and the regenerating cilium in the sea urchin embryo.

Methods and Materials

Embryos of *Arbacia punctulata* were maintained in natural seawater; embryos of *Strongylocentrotus purpuratus* were kept in artificial seawater (Instant Ocean). Eggs and sperm were shed by KCl injection or electrical stimulation (Harvey, 1956). After insemination development was allowed to proceed at 18° C for both species.

Deciliation was performed according to Auclair and Siegel (1966). Hypertonic seawater treatment (29.2 g NaCl/liter of seawater for 1–2 min) was found to remove essentially all cilia. Following deciliation embryos were resuspended in normal seawater for regeneration to occur.

The initial stages of regeneration were observed and measured after staining with Lugol's iodine. Measurements on cilia longer than 6–8 μ were made on living

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specimens (in 5% methyl cellulose) using phase contrast optics. The longest observable cilium on each of five embryos was measured and the values averaged.

The temperature-control housing, pressure pump and microscope-pressure chamber were similar to those described by Marsland (1950). Following deciliation the embryos at the stage of swimming blastula or early prism were transferred to a Lucite chamber which was then placed inside the main pressure vessel. In all regeneration experiments, the pressure (2000–10,000 psi, which is equivalent to 139–680 atm, or 137.8×10^5–689×10^5 Newtons/m^2 or Pascals) was applied 3 min after deciliation. Non-pressurized control embryos were placed in similar chambers but remained at atmospheric pressure. The embryos were observed at magnifications up to 600 × while the cells were under pressure.

Results

Preliminary observations

The pattern of behavioral changes induced by pressure on swimming embryos of *Strongylocentrotus purpuratus* and *Arbacia punctulata* is dependent upon both magnitude and duration of treatment. The changes induced at the higher pressures are quite clear whereas at lower magnitudes of pressure there is considerable individual variation within a single group.

Strongylocentrotus embryos (swimming blastulae) were subjected to varying magnitudes of pressure (6000–10,000 psi) while under continuous observation. Within two minutes after compression to 10,000 psi, the embryos began to slow down, although they still maintained their normal spiralling motion. Following 3–5 min of compression the embryos began to collect on the bottom of the pressure chamber where they rotated with one pole against the surface. The rate of spinning gradually slowed and was replaced by a very rapid vibratory motion. After 7–8 min most of the embryos were vibrating, however a few embryos retained their spinning motion and a few were completely motionless. After 10–12 min essentially all of the embryos were motionless.

When the pressure was lowered to 8000 psi the cells responded in a similar fashion to that found at 10,000 psi except that some motion was evident 15 min after compression. At 7000 psi it took considerably longer for the embryos to display the previously described effects. After 10 min at 7000 psi most of the embryos were still swimming, albeit more slowly than normal, and only a few embryos had settled to the bottom of the chamber. By 20 min all of the embryos had settled and were rotating slowly. Thirty min after compression, the embryos were still rotating although they were beginning to show signs of disaggregation. For this reason, longer durations were not employed at this pressure level. With a further reduction in pressure to 6000 psi it required between 20 and 30 min for most of the embryos to settle on the bottom of the chamber. At 50–60 min after compression, most of the embryos were still rotating. It was felt that the extent of disaggregation limited the usefulness of experiments of longer duration than 60 min.

In experiments in which the pressure was released while the embryos were still active (*i.e.*, displaying some movement) an initial burst of activity followed immediately upon decompression.

It was generally found that *Arbacia* embryos displayed similar behavior; however, they were more sensitive to pressure treatment than *Strongylocentrotus* embryos.

Pressure effects on sea urchin cilia

From the above study it was evident that high pressure treatment resulted in a loss of cilia. In order to investigate this phenomenon, *Strongylocentrotus* embryos were subjected to pressure and following decompression they were studied by phase contrast microscopy. The number of cilia lost depended partly on the magnitude and partly on the duration of treatment. At pressures of 8000 psi and above more than 90% of the cilia were removed within 10 min; at 7000 psi more than 80% of the cilia were removed 30 min after compression. When the pressure was reduced to 6000 psi more than 50% of the cilia were lost in 45–60 min. The cilia were lost as apparently intact structural units and could be observed floating in the medium. The detached cilia had distended bulbous distal tips similar to the cilia which remained on the embryos.

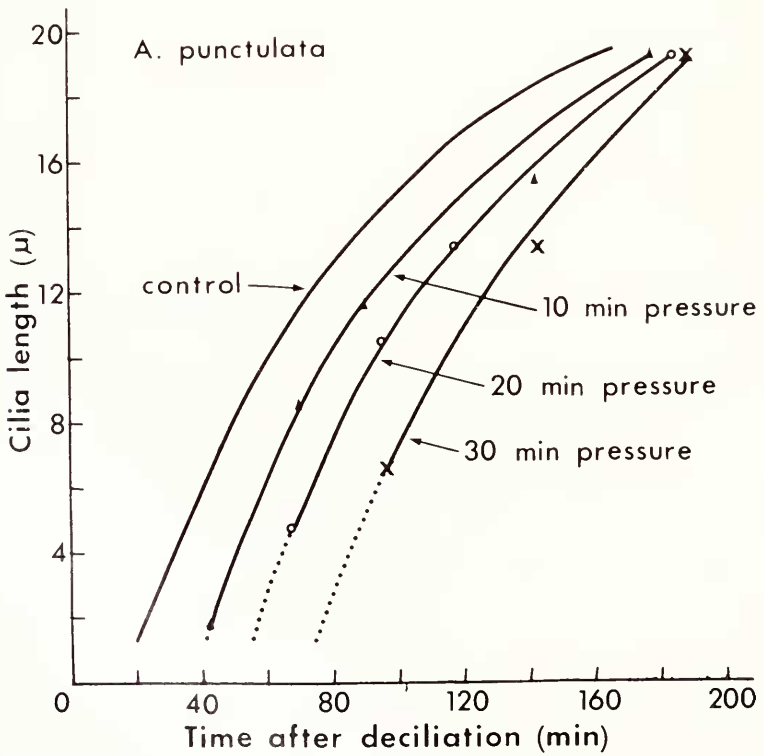

FIGURE 1. Duration of pressure treatment and cilia regeneration in *Arbacia punctulata*. Sea urchin prismatic stage larvae were deciliated and subjected to 10,000 psi for 10, 20 and 30 min of compression. At various times after decompression the lengths of the cilia were measured.

In a series of experiments at 10,000 psi, in which groups of embryos were treated for varying durations (2–10 min), it was found that a few cilia remained on those embryos still undergoing the vibratory motion and essentially all cilia were gone from those embryos in which all motion had ceased.

In embryos treated for long durations (30–60 min) at lower pressures (6000 psi) the apical tuft cilia were found to be more resistant to pressure than other cilia. In embryos displaying partial cilia loss, the deciliation frequently occurred in a patch near the vegetal pole.

Comparable studies with *Arbacia* embryos indicated that they were more sensitive to pressure-induced cilia loss than *Strongylocentrotus* embryos. In some experiments, pressures as low as 2000 psi for durations of less than one hour caused essentially total loss of cilia from *Arbacia* embryos.

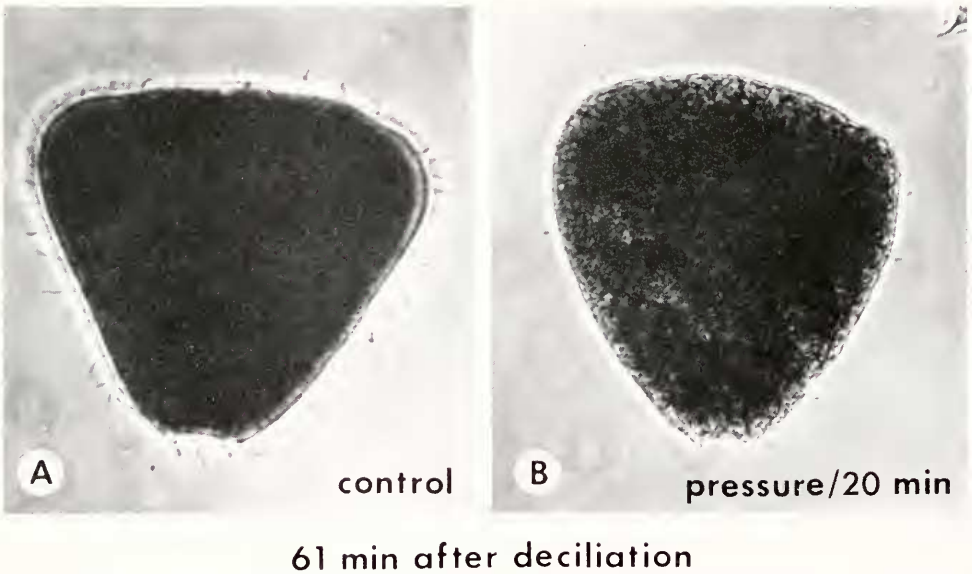

FIGURE 2. Pressure induced delay of cilia regeneration. The photomicrographs were taken 61 min after deciliation by hypertonic seawater; (A) Control embryo; (B) Experimental embryo was subjected to 10,000 psi for 20 min. Note the rounded appearance of the surface cells. Both control and experimental embryos were stained with Lugol's iodine.

Pressure effects on cilia regeneration

Since it is well established that high pressure interferes with microtubule polymerization, the effects of pressure on cilia regeneration were investigated. The two parameters studied were the magnitude and the duration of pressure treatment. Embryos were deciliated by hypertonic sea water treatment and subjected to compression (2000–10,000 psi) for varying durations (10, 20, or 30 min). Following decompression, the lengths of the regenerating cilia were recorded and compared to controls. The regeneration delay for *Arbacia* embryos was found to be a function of the duration of pressure treatment (Fig. 1). The onset of regeneration was delayed for 10–25 min in excess of the duration of compression. This delay

was proportional to the duration of treatment. It was not possible to ascertain from this data whether or not the rate of cilia regeneration was affected by the pressure treatment. Photomicrographs of representative experimental (10,000 psi for 20 min) and control embryos 61 min after deciliation are shown in Figure 2. The cilia in the pressure treated embryo measure 4 μ as compared to the control cilia which are 11 μ. The surface cells of pressurized embryos tend to round up during treatment. When the cilia had regenerated to a length of a few microns, ciliary motion was readily evident.

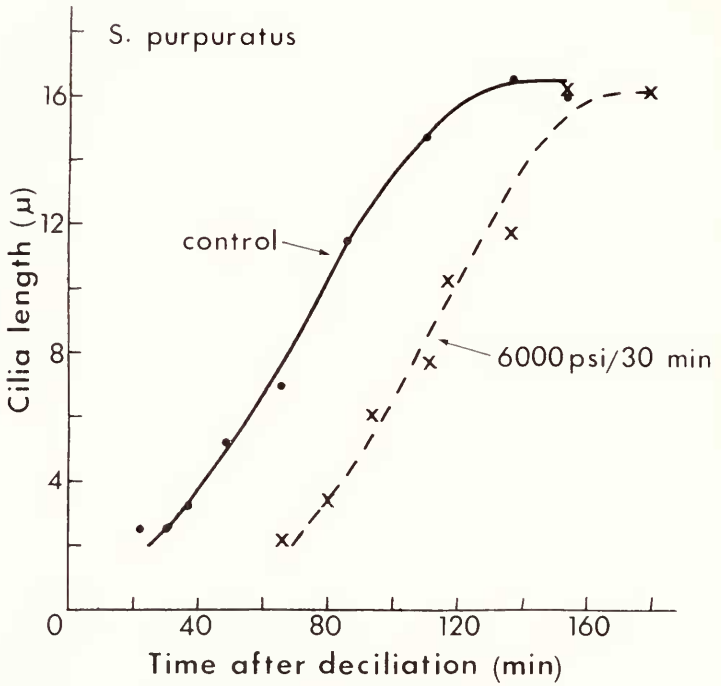

FIGURE 3. Effects of pressure on cilia regeneration in *Strongylocentrotus purpuratus* embryos. Embryos at the hatched blastula stage were subjected to 6000 psi for 30 min immediately after deciliation with hypertonic seawater. Cilia regeneration of control (•——•) and pressure treated cells (x- - - -x) are illustrated.

A similar pattern of regeneration was found for *Strongylocentrotus* swimming blastulae. The regeneration kinetics for a representative series of embryos subjected to 6000 psi for 30 min is shown in Figure 3. The regeneration delay for this pressure-duration treatment was 10 min in excess of treatment. This data suggests that the rate of regeneration was not affected by the pressure treatment.

The effects of various magnitudes of pressure on cilia regeneration was investigated in *S. purpuratus*. The duration was kept constant (30 min) but the magnitude of pressure was varied systematically. In Figure 4 the cilia regeneration curves at three different pressure levels (2000, 4000, and 5000 psi) are shown. The regeneration delay was related to the applied pressure. At lower pressures the

cilia were able to regenerate under compression. Short cilia were visible immediately following 30 min compression at 2000 and 4000 psi. At 5000 psi there was no regeneration during compression. As shown in Figure 4, following the release of pressure the regeneration profiles at the three pressure levels were comparable to controls.

Discussion

These studies show that high hydrostatic pressure is capable of profoundly affecting the motility of sea urchin embryos and eventually results in a loss of cilia. In experimentally deciliated embryos, cilia regeneration is blocked at high pressures or retarded at lower pressures for the duration of the treatment.

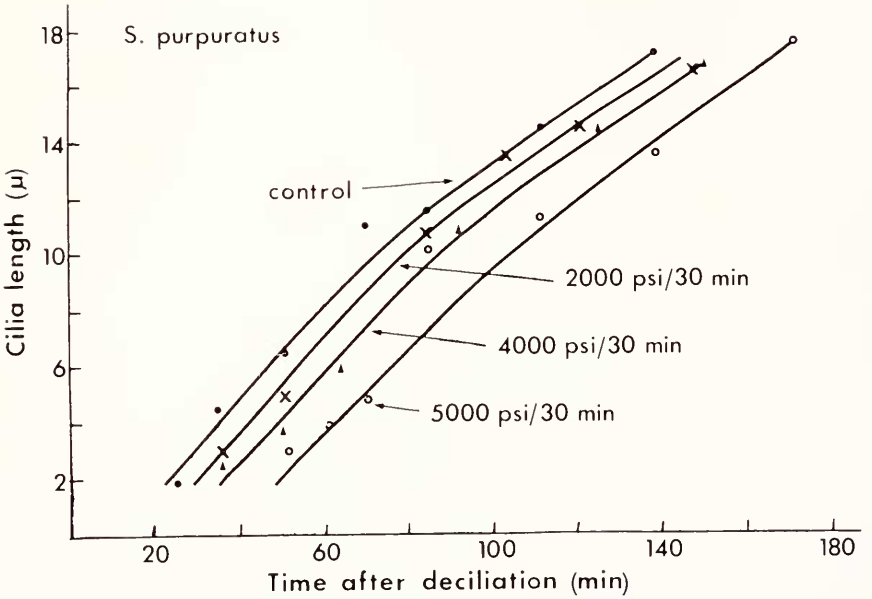

Figure 4. Effects of pressure on regeneration in *Strongylocentrotus purpuratus*. Immediately following deciliation by hypertonic seawater, the hatched blastulae were subjected to different magnitudes of pressure (2000, 4000 or 5000 psi) for a standardized duration of 30 min. Cilia length was plotted as a function of time after deciliation.

Relatively few observations have been reported concerning pressure effects on ciliary activity in sea urchin embryos although considerable work has been done on other organisms such as protozoa (Kitching, 1957 and 1970; Kennedy and Zimmerman, 1970). In general, high pressure inhibits the activity of cilia. Tilney and Gibbins (1968, 1969) have reported in *Arbacia* embryos that pressure (6000–7000 psi) results in a slowing or stopping of locomotion and progressive cell disaggregation. However, they did not report any ciliary loss at these pressures for durations up to three hours. This is perhaps explained by the fact that their primary interest was the analysis of fine structure and partial ciliary loss may not

have been apparent. They reported that the microtubular elements, the basal body, and rootlet of the cilium were unaffected by pressures of 6000–7000 psi. *Tetrahymena* cilia, however, are quite sensitive to pressure treatment (Kennedy and Zimmerman, 1970). Pressures of 7500 or 10,000 psi induced a degradation of the central ciliary microtubules and a disorganization of longitudinal microtubules after durations of only 2 to 10 min. The lack of observable ultrastructural effect on sea urchin cilia may perhaps reflect a basic difference in ciliary structure from that found in *Tetrahymena*, although this is not readily apparent. The present studies indicate that sea urchin cilia (or associated structures) are affected by pressures in excess of 6000 psi, at least in the region of the base of the cilium, since pressure induces the loss of cilia as apparently intact units. The significance of the bulbous tips observed on detached cilia following pressure treatment is not known, although such tips have been observed in cilia removed by other means (Auclair and Siegel, 1966). In flagellae a bulbous tip can be produced by the rolling up of the axoneme inside the flagellar membrane (Rosenbaum and Child, 1967).

The mechanism by which pressure induces cilia loss is not known. On the basis of the present study it is difficult to speculate since even the precise level of amputation is in doubt. It is possible that the cytoplasmic microtubules near the basal body and/or the cortical plasmagel may be involved in anchoring the cilium since these structures have been shown to be pressure sensitive (Tilney and Gibbins, 1968 and 1969; Marsland, 1970). Recently Blum (1972) has proposed that there is a specialized breaking point in the transitional region which lies between the kinetosome and the ciliary shaft.

It has been convincingly demonstrated (Auclair and Meismer, 1965; Auclair and Siegel, 1966) that initial cilia formation and regeneration do not depend on RNA or protein synthesis in sea urchin embryos. This implies that a considerable pool of the necessary proteins exists in the cells (Auclair and Siegel, 1966). More recent work utilizing pactamycin as a protein synthesis inhibitor indicates that protein synthesis may be necessary for regeneration (Child and Apter, 1969). This delay in regeneration may, however, be due to non-specific effects of the drug since earlier studies had shown that puromycin did not block regeneration although 89% of cellular protein synthesis was inhibited (Auclair and Siegel, 1966). Inhibition of regeneration by pressure, therefore, is probably through interference with assembly processes, possibly the polymerization of the microtubules. It has already been well established that pressure is capable of depolymerizing and preventing the reformation of microtubules in the cytoplasm (Tilney and Gibbins, 1968 and 1969), the mitotic apparatus (Zimmerman and Marsland, 1964) and the axopodia of Heliozoa (Tilney *et al.*, 1966). Although the intact cilium in the sea urchin appears to be insensitive to pressure as regards to depolymerization it is clear from the data that cilia formation is pressure sensitive and that growth rate can be slowed at lower pressures or totally inhibited at higher ones. This may be a result of a shift in a dynamic equilibrium between polymerized and free microtubular subunits. The reason for the excess delay is unknown although it probably reflects reparable cellular damage of some type.

Summary

The effects of hydrostatic pressure on the cilia of sea urchin embryos (*Arbacia*

and *Strongylocentrotus*) were investigated. At a pressure of 10,000 psi the swimming blastula and early gastrula embryos became less active. They lost their translational movement and began to rotate slowly on the bottom of the chamber; in about 10 min all movement stopped and essentially all cilia had fallen from the embryos. At lower pressures and with longer durations the embryos were differentially affected and there was considerable variation in the number of cilia removed from individual embryos. With pressures of 6000 psi for 60 min the majority of the embryos lost more than 50% of their cilia. *Arbacia* embryos were more pressure-sensitive than *Strongylocentrotus* embryos.

Following deciliation with hypertonic seawater, hydrostatic pressure above 5000 psi was found to block regeneration for the duration of the pressure treatment. At 6000 psi and above the regeneration delay was in excess of the duration of pressure treatment; the regeneration delay was directly proportional to the duration of treatment. At pressures lower than 5000 psi sea urchin cilia were able to regenerate under pressure but at a reduced rate relative to controls. Pressure treatment does not affect regeneration rate following decompression.

The results are discussed in terms of the known effects of pressure on cellular systems.

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